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Response of high yield variety of Mung bean (*Vigna radiata* L.) after fertigation with distillery effluent in two seasons

Vinod Kumar* and A.K. Chopra

*Agro-ecology and Pollution Research Laboratory, Department of Zoology and Environmental Science. Gurukula Kangri University, Haridwar-249404 (Uttarakhand), India.

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A pot experiment was conducted to study the response of high yield variety of *Vigna radiata* (Mung bean; Var. Pusa-105), after fertigation with distillery effluent (DE) in two seasons, that is, rainy and summer season. The DE irrigation produced significant (P<0.001) changes in electrical conductivity (EC), pH, organic carbon (OC), sodium (Na⁺), potassium (K⁺), calcium (Ca²⁺), magnesium (Mg²⁺), iron (Fe²⁺), total Kjeldahl nitrogen (TKN), phosphate (PO₄³⁻), sulphate (SO₄²⁻), zinc (Zn), copper (Cu), cadmium (Cd), manganese (Mn) and chromium (Cr) of the soil. The non-significant (P>0.05) changes were observed in water holding capacity (WHC) and bulk density (BD) of the soil in both seasons. The agronomical performance of *V. radiata* was gradually increased from 5 to 50% concentrations of DE in the rainy season and 5 to 25% concentration of DE in the summer season, while it was decreased at higher concentration, that is, from 75 to 100% concentrations of DE in the rainy season and 50 to 100% concentrations of DE in the rainy season and at 25% in the summer season. The contamination factor (Cf) of various metals were in order of Mn>Cd>Cr>Cu>Zn for soil and Cr>Cu>Zn>Cd>Mn for *V. radiata* in both seasons after irrigation with DE.

Key words: Distillery effluent, Vigna radiata (Mung bean), fertigation, metals, rainy and summer seasons, contamination factor.

INTRODUCTION

The discharge of effluents, treated or untreated, into the environment, particularly in the aquatic bodies such as lakes, rivers and the coastal marine environments can cause severe degradation of these aquatic ecosystems (Bharagava et al., 2008; Chandra et al., 2009; Kumar and Chopra, 2012). Therefore, use of effluent is one of the many options being considered as a source of water in regions of water scarcity (Ramana et al., 2002; Biswas et al., 2009; Tharakeshwari and Jagannath, 2011a). The growing demand of water for irrigation has resulted in a marked increase in the use of treated or untreated wastewater worldwide (Hati et al., 2007; Kalaiselvi et al., 2009; Tharakeshwari and Jagannath, 2011a). Distilleries are one of the most important agro-based industries, which produce ethanol from molasses for the preparation of wine, and other alcoholic beverages (AIDA, 2004). These generate huge volumes of foul smelling and dark brown colored wastewater known as spent wash (Biswas et al., 2009; Kumar and Chopra, 2010). The effluents contain substantial amount of organic matter and nutrients especially potassium and sulphur whichmay be

ABBREVIATIONS: ANOVA, Analysis of variance; **BIS,** Bureau of Indian standards; **HI,** Harvest index; **LAI,** Leaf area index.

^{*}Corresponding author. E-mail: drvksorwal@gmail.com. Tel: +91-1334 249091.

beneficial for the growth of plants, and thus provide a potentially useful alternative for the safe disposal of spent wash (Kumar and Chopra, 2012). In India, there are about 330 distilleries with total installed capacity of about 3500 million liters of alcohol (AIDA, 2004). Distillery industries play major role in the environment pollution, generate a huge volume of effluents (spent wash) with enormous quantity of organic and inorganic nutrients in the form of very high BOD, COD, TKN, Na, K, Ca, Mg and other nutrients (Joshi et al., 2000; Hati et al., 2007; Kumar and Chopra, 2012).

Furthermore, effluents from these industries contain appreciable amounts of organic carbon, sodium, potassium, calcium, magnesium metallic cations like zinc, copper, iron, manganese, lead, nickel and cadmium (Chopra et al., 2009; Tharakeshwari and Jagannath, 2011a). In the agriculture, irrigation water quality is believed to have effects on the soil characteristics, and growth of agricultural crops (Shainberg and Oster, 1978). Long term irrigation with effluents increases organic carbon content and metals accumulation in soil and increase the chances of their entrance in food chain (Bharagava et al., 2008; Biswas et al., 2009; Chopra et al., 2009).

Vigna radiata (Mung bean) is cultivated globally in tropical and subtropical countries. In India, it is grown two times in a year in two seasons, one sown in the early period of March for summer crop, and in the early period of July for rainy crop (Aggarwal et al., 1992). It is used as green vegetable pulse, medicinal purposes and cattle fodder also (Lawn and Ahn, 1985; Aggarwal et al., 1992; Mubarak, 2005).

The utilization of industrial effluent for irrigation of agricultural crops has generated interest in recent times (Tharakeshwari and Jagannath, 2011a; Kumar and Chopra, 2013). Most crops give higher potential yields with effluent irrigation; reduce the need for chemical fertilizers, resulting in net cost savings to farmers (Ramana et al., 2002; Kalaiselvi et al., 2009; Kumar and Chopra, 2013). However, industrial effluents may cause enrichment of metals in the top soil if it is regularly applied to soil in excessive amount, and ultimately may reduce yield and impure the quality of crops (Hati et al., 2007; Bharagava et al., 2008; Kumar and Chopra, 2010). Thus, it is important to understand the crop-effluent relationship, for their appropriate application in irrigation practices (Kaushik et al., 2005). In recent past, various studies have been made on the characteristics of effluent of industries, and their effect on agronomical characteristics of various crop plants (Ramana et al., 2002; Hati et al., 2007; Pandey et al., 2007; Bharagava et al., 2008; Chandra et al., 2009; Kalaiselvi et al., 2009; Kumar and Chopra, 2013). Chandrasekar et al. (1998) reported that the germination and seedling growth of black gram (Vigna mungo L.) was decreased when the concentration of sugar mill effluent increased. Kannan and Upreti (2008) studied the effect of distillery effluent

on germination and growth of V. radiata (L.). Tharakeshwari and Jagannath (2011a) observed the response of seedling growth of V. radiata (L.) to distillery effluent. Thus, most of these studies have been conducted with fewer agronomical stages, with limited parameters of various plants, but there are very few comprehensive reports with all agronomical stages of these plants (Kannan and Upreti, 2008; Tharakeshwari and Jagannath, 2011a). Therefore, much attention has not been paid for the use of distillery effluent for irrigation of V. radiata in different seasons. Keeping the above in view, the reuse of this agro-based industrial effluent and economic importance of V. radiata, the present investigation was undertaken to study the response of V. radiata (L.) after fertigation with distillery effluent in two seasons.

MATERIALS AND METHODS

Experimental design

A pot experiment was conducted in the Experimental Garden of the Department of Zoology and Environmental Sciences, Faculty of Life Sciences, Gurukula Kangri University Haridwar (29°55'10.81" N and 78°07'08.12" E). The plants of V. radiata were grown in early March for summer crop, and in early July for the rainy season crop during the period of 2008 and 2009. The experiment was conducted under completely randomized design (pots were placed randomly for various treatments), and replicated six times in each season. The number of pots (42) having soil were used for the cultivation of V. radiata. Proper distance was maintained between each replicate (30 cm), between each treatment (60 cm), and plant to plant (5 cm), for the maximum performance of the crop. Each poly bag was made porous for aeration, and it was labeled for the various treatments namely 0 (control, bore well water), 5%, 10%, 25%, 50%, 75%, and 100%.

Effluent collection and analysis

Doon distillery Dehradun (Uttarakhand) was selected for the collection of effluent samples. The samples of DE were collected in plastic containers from the outlet of a settling tank installed by the distillery used to reduce the BOD and solids. The effluent samples were brought to the laboratory, and was analyzed for various physicochemical and microbiological parameters namely: total dissolved solids (TDS), pH, electrical conductivity (EC), dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD), chlorides (CI), bicarbonates (HCO₃⁻), carbonates (CO₃²⁻), sodium (Na⁺), potassium (K^+), calcium (Ca²⁺), magnesium (Mg²⁺), total Kjeldahl nitrogen (TKN), nitrate (NO₃²⁻), phosphate (PO₄³), sulphate (SO₄²), iron (Fe), zinc (Zn), cadmium (Cd), copper (Cu), chromium (Cr), manganese (Mn), standard plate count (SPC) and most probable number (MPN),

following standard methods (APHA, 2005; Chaturvedi and Sankar, 2006).

Soil preparation, filling of pots, sampling and analysis

The soil used for cultivation was collected at a depth of 0 - 15 cm. Each pot (30×30 cm) was filled with 5 kg well prepared soil, earlier air-dried and sieved to remove debris, and mixed with equal quantity of farmyard manure. The plants were irrigated twice in a week with 500 ml of DE concentrations along with bore well water (control). The soil was analyzed after DE irrigation for various physico-chemical namely: moisture content, bulk density, WHC, EC, pH, OC, Na⁺, K⁺, Ca²⁺, Mg²⁺, Fe²⁺, TKN, PO₄³⁻, SO₄²⁻, Zn, Cd, Cu, Cr and Mn following standard methods cited in Chaturvedi and Sankar (2006).

Sowing of seeds, irrigation pattern and collection of crop parameter data

The seeds of a high yielding variety of V. radiata (Var. Pusa-105) were procured from ICAR, Pusa, New Delhi, and sterilized with 0.01% mercuric chloride, and was soaked for 12 h. Seven seeds were initially sown in each pot at equal distance between plant to plant (7.5 cm). Five plants were maintained in each pot by thinning out of the germinated plants. The plants were irrigated with 500 ml of their respective concentration namely: 5%, 10%, 25%, 50%, 75% and 100% of DE along with control. The plants were irrigated twice in a week/as per the requirement of crop plants, and no drainage was allowed. The various agronomical parameters of V. radiata at germination (0-15 days) to maturity stages (90 days) namely: seed emergence, seed germination, shoot length, root length, number of flowers, number of pods, and crop yield, were determined following standard methods (Chandrasekar et al., 1998), dry weight (Milner and Hughes, 1968) and chlorophyll content (Porra, 2002). The relative toxicity (Pandey et al., 2008), LAI (Denison and Russotti, 1997) and HI (Sinclair, 1998) was calculated as follows:

Relative toxicity:

Relative toxicity (RT) = $\frac{\text{Germination percentage with control}}{\text{Germination percentage with effluent}} \times 100$

Leaf area index:

Leaf area index (LAI) = $\frac{\text{Leaf area}}{\text{Land area}}$

Harvest index:

Harvest index (HI) = $\frac{\text{Grain weight (g.)}}{\text{Total plant weight (g.)}} \times 100$

Digestion of samples for metals analysis

For metals analysis, 10 ml sample of effluent, 1.0 g sample of air dried soil/plant was taken in digestion tube separately. For each sample, 3 ml concentrate of HNO_3 was added, and digested on the electrically heated block for 1 h at 145°C. Then 4 ml of $HCIO_4$ was added, and heated to 240°C for an additional hour. The aliquot was cooled and filtered through Whatman # 42 filter paper, and make up the volume of 50 ml, and used for analysis. The metals were analyzed by using atomic absorption spectrophotometer (PerkinElmer, Analyst 800 AAS, Gen Tech Scientific Inc., Arcade, NY) following standard methods (APHA, 2005; Chaturvedi and Sankar, 2006). The contamination factor (Cf) for metals accumulated in DE irrigated soil, and *V. radiata* were calculated by following the formula cited in Håkanson (1980):

Contamination factor (Cf) = $\frac{\text{Mean content of metal in the sample}}{\text{Background metal content of the substance}}$

Statistical analysis

Data were analyzed with SPSS (ver. 12.0, SPSS Inc., Chicago, III.). Data were subjected to two-way ANOVA and Duncan's multiple range test was also performed to determine whether the difference was significant or non significant. Mean standard deviation and coefficient of correlation (r-value) of soil and crop parameters with effluent concentrations were calculated with MS Excel (ver. 2003, Microsoft Redmond Campus, Redmond, WA) and graphs were produced with Sigma plot (ver. 12.3, Systat Software, Inc., Chicago, IL).

RESULTS AND DISCUSSION

Characteristics of distillery effluent

The characteristics of Doon distillery effluent (spent wash) are given in Tables 1 and 2. The results revealed that the effluent was acidic in nature, that is, pH 5.67. The low pH of DE might be due to the presence of higher concentration of organic acids such as CH₃COOH. Among various parameters, BOD, COD, Cl, Ca²⁺, TKN, $NO_3^{2^-}$, $SO_4^{2^-}$, MPN and SPC were found beyond the prescribed limit of Indian Irrigation Standards (BIS, 1991). High BOD, COD, TKN, $NO_3^{2^2}$ and $PO_4^{3^2}$ might be due to the presence of high oxidizable organic matter, and rapid consumption of dissolved inorganic materials. The content of HCO_3^- , $CO_3^{2^-}$, Na^+ , K^+ , Mg^{2+} and $SO_4^{2^-}$ were also found slightly higher in DE, which is associated with higher EC of the effluent (Tables 1 and 2). The findings were very much in accordance with those of Kumar and Chopra (2012). In case of metals, the content of Fe^{2+} , Zn, Cd, Cu, Cr and Mn were also recorded significantly higher in DE than the permissible limit given by the Bureau of Indian Standard (Tables 1 and 2). The content

Deserveden			Efflu	ent concentr	ation (%)			BIS ^b for
Parameter	0 (BWW) ^a	5	10	25	50	75	100	irrigation water
TDS (mg L ⁻¹)	221.5	478.54*	860.00**	1964.85**	2413.00***	4396.84***	7680.00***	1900
EC (dS m ⁻¹)	0.31	0.74*	1.37**	3.15**	3.81***	6.97***	12.68***	- ^c
рН	7.52	7.56ns	7.41ns	7.04ns	6.72ns	6.35ns	5.67ns	5.5-9.0
DO (mg L ⁻¹)	8.24	6.36ns	4.68*	4.87**	2.42**	1.18**	NIL	-
BOD (mg L ⁻¹	3.84	164.60*	334.27**	850.5**	1635.5***	2462.52***	3265.58***	100
COD (mg L ⁻¹)	5.95	448.40*	897.81**	2231.0**	4352.5***	6495.54***	8653.02***	250
Cl ⁻ (mg L ⁻¹)	15.78	105.34*	185.54**	460.57**	859.04***	1248.08***	1653.77***	500
HCO ₃ ⁻ (mg L ⁻¹	282.06	585.60*	650.52**	834.52**	1389.59***	1884.57***	2254.53***	-
CO3 ²⁻ (mg L ⁻¹)	105.85	165.57*	188.80**	227.56**	245.51***	285.35***	356.57***	-
Na⁺ (mg L⁻¹)	9.76	14.30*	27.50**	69.04**	138.80***	217.06***	277.02***	-
K⁺ (mg L ⁻¹)	5.54	35.40*	61.45**	154.55**	278.90***	408.82***	536.56***	-
Ca ²⁺ (mg L ⁻¹	23.53	135.60*	213.33**	480.07**	953.20***	1425.03***	1855.05***	200
Mg^{2+} (mg L ⁻¹)	12.27	37.53*	46.80**	96.54**	159.50***	231.08***	284.07***	-
TKN (mg L ⁻¹)	24.31	60.67*	75.60**	136.02**	3207***	456.07***	572.54***	100
NO_3^{2-} (mg L ⁻¹)	25.28	117.55*	173.34**	430.50**	760.30***	1139.53***	1455.36***	100
PO4 ³⁻ (mg L ⁻¹)	0.04	32.06*	66.53**	168.07**	323.07***	476.57***	637.55***	-
SO4 ²⁻ (mg L ⁻¹)	17.61	96.55*	155.37**	298.53**	633.80***	954.52***	1246.09***	1000
Fe ²⁺ (mg L ⁻¹)	0.34	1.54*	3.18**	7.86**	15.37***	22.88***	30.59***	1.0

Table 1. Physico-chemical characteristics of distillery effluent (DE).

"Least square mean analysis; Significant F -***P >0.1% level, **P> 1% level; *P> 5% level; ns- Not significant; aWW = well water control; bBIS = Bureau of Indian Standard; c "-" = No standard available" in continuous manner.

Table 2. Heavy metals and microbiological characteristics of distillery effluent (DE).

			Efflu	uent concen	tration (%)			BIS ^b for
Parameter	0 (BWW) ^a	5	10	25	50	75	100	irrigation water
Zn (mg L ⁻¹)	0.06	0.31**	0.62**	3.18**	6.24***	9.29***	20.0***	15
Cd (mg L ⁻¹)	0.10	0.13*	0.16**	0.34**	0.67***	0.99***	2.3***	2.00
Cu (mg L ⁻¹)	0.04	0.11**	0.24**	0.43**	0.86***	1.29***	4.72***	3.00
Mn (mg L ⁻¹)	0.02	0.09*	0.18**	0.45**	0.91***	1.36***	3.81***	1.00
Cr (mg L ⁻¹)	0.04	0.05*	0.07**	0.18**	0.36***	0.54***	2.72***	2.00
SPC (SPC mL ⁻¹)	6.3×10 ¹	3.8×10 ⁴ *	5.3×10 ⁵	7.4×10 ⁶	4.6×10 ⁷ ***	2.4×10 ⁸ ***	3.6 ×10 ¹⁰ ***	10000
MPN (MPN100 mL ⁻¹)	2.6×10 ¹	4.9×10 ³ *	6.8×10 ³	8.4×10 ³	4.6×10 ⁴ ***	6.6×10 ⁵ ***	4.6×10 ⁶ ***	5000

"Least square mean analysis; Significant F -***P >0.1% level, **P> 1% level; *P> 5% level; aWW = well water control; bBIS = Bureau of Indian Standard in continuous manner.

of metals was also recorded higher in distillery effluent as earlier reported by Chandra et al. (2009).

Characteristics of soil

The soil characteristics after irrigation of *V. radiata* with different concentrations of DE in both the rainy and summer seasons are presented in Tables 3 to 7. The results showed that no drastic change was observed in the soil texture (loamy, sand: silt: clay: 60: 20: 20) with the application of all concentrations of DE throughout the period of trial.

The present study showed that the soil irrigated with

100% DE showed increase in EC (88.51-106.63%), OC (3813.95-4215.21%), Na⁺ (273.87-276.12%), K⁺ (69.96-75.79%), Ca²⁺ (1191.21-1221.97%), Mg²⁺ (8379.41-9389.59%), Fe²⁺ (450.56-557.46%), TKN (1336.66-1346.50%), PO₄³⁻ (329.83-340.66%), SO₄²⁻ (130.26-154.56%), Zn (398.73-439.50%), Cd (2100.00-2245.45%), Cu (439.10-459.51%), Mn (2561.11-2973.68%) and Cr (1661.11-1964.28%), and decrease in moisture content (18.84-22.69%), WHC (13.26-15.61%), BD (1.40%), and pH (16.51-17.52%) of the soil in comparison to bore well water (control) irrigated soil in both seasons (Tables 3 to 7). A significant increase in the OC, Na⁺, K⁺, Ca²⁺, Mg²⁺, Fe²⁺, TKN, PO₄³⁻, SO₄²⁻, Zn, Cd, Cu, Mn and

%DE × Season	Soil mo	isture (%)	WH	C (%)	BD (g	m cm ⁻³)	EC (EC (dS m ⁻¹)	
%DE x Season	Rainy	Summer	Rainy	Summer	Rainy	Summer	Rainy	Summer	
0 (Control)	61.08	61.08	45.98	45.98	1.42	1.42	2.09	2.11	
5	60.82	58.64	45.31	44.15	1.42	1.42	2.43	2.85	
10	56.61	54.84	44.22	43.35	1.42	1.42	2.72a	2.95a	
25	53.83	51.23	42.79	41.56	1.41	1.41	2.91a	3.13a	
50	51.87	49.56	41.82	40.84	1.41	1.41	3.23a	3.66a	
75	50.27	48.24	40.85	39.65	1.40	1.40	3.48a	3.93a	
100	49.57	47.22	39.88	38.80	1.40	1.40	3.94a	4.36a	
CD	6.26***	7.65**	1.16ns	2.59ns	0.52ns	0.37ns	33.08**	26.49***	
F-calculated	7.49	6.77	5.17	5.42	0.2	0.21	0.31	0.35	

Table 3. Change in physical parameters of soil after irrigation of *V. radiata* with DE till harvesting (90 days after sowing) in both seasons.

Least square mean analysis; Significant F -***P >0.1% level, **P> 1% level; a - significantly different to the control; CD- Critical difference; ns - Not Significant.

Table 4. Change in pH, OC and TKN of soil after irrigation of V. radiata with DE till harvesting (90 days after sowing) in both seasons.

%DE × Season	р	Н	OC(mg	∣ Kg ⁻¹)	TKN(mg Kg ⁻¹)		
%DE x Season	Rainy	Summer	Rainy	Summer	Rainy	Summer	
0 (Control)	7.51	7.53	0.43	0.46	32.21	33.46	
5	7.48	7.50	1.00	1.02	59.88a	66.13a	
10	7.30	7.23	1.96a	2.18a	75.75a	87.00a	
25	7.23	7.01	5.16a	5.59a	145.33a	156.58a	
50	6.66a	6.58a	9.82a	11.84a	286.43a	297.68a	
75	6.31a	6.24a	14.80a	16.82a	406.75a	418.00a	
100	6.27a	6.21a	16.83a	19.85a	462.75a	484.00a	
CD	6.1***	5.71**	201.06***	202.89***	4567.37***	5755.18***	
F-calculated	0.64	0.66	1.4	1.39	6.8	7.36	

Least square mean analysis; Significant F -***P >0.1% level, **P> 1% level; a - significantly different to the control; CD- Critical difference.

Table 5. Changes in cations of soil after irrigation of V. radiata with DE till harvesting (90 days after sowing) in both seasons.

	Na⁺ (m	ng Kg ⁻¹)	K⁺(mg Kg ⁻¹)		Ca ²⁺ (mg Kg ⁻¹)		Mg ²⁺ (mg Kg ⁻¹)		Fe ²⁺ (mg Kg ⁻¹)	
%DE× Season	Rainy	Summer	Rainy	Summer	Rainy	Summer	Rainy	Summer	Rainy	Summer
0 (Control)	18.81	20.06	155.34	156.59	15.36	16.61	1.70	1.73	2.65	2.68
5	24.06	27.31	161.88	169.14	20.40	25.65	3.45	3.98	3.16	3.96
10	29.47	31.72	173.09a	179.34a	28.81a	34.06a	5.45	6.48	5.66	6.68
25	31.35a	35.60a	225.80a	237.05a	113.79a	125.04a	21.07a	29.09a	7.78a	8.81a
50	37.53a	41.78a	241.22a	252.47a	141.17a	152.42a	58.97a	65.99a	9.74a	10.76a
75	51.09a	58.34a	255.31a	262.56a	183.28a	194.53a	116.78a	129.80a	11.73a	13.76a
100	70.75a	75.00a	264.02a	275.27a	198.33a	219.58a	144.15a	164.17a	14.59a	17.62a
CD	48.21***	46.55***	84.65***	120.32***	607.18***	585.82***	28.28***	29.05***	29***	29.17***
F-calculated	7.3	7.43	9.33	7.83	6.83	6.95	4.47	4.49	1.64	1.63

Least square mean analysis; Significant F -***P >0.1% level; a - significantly different to the control; CD- Critical difference.

Cr content of the soil might be attributed to higher organic load of DE. Similarly, earlier studies of soil irrigated with DE also showed an increase for these parameters as reported by Kumar and Chopra (2012).

%DEx Season	PO4 ³⁻ (m	lg Kg ⁻¹)	SO4 ²⁻ (mg Kg ⁻¹)			
%DEx Season	Rainy	Summer	Rainy	Summer		
0 (Control)	53.00	54.25	74.37	75.62		
5	58.64	62.89	80.34	86.59		
10	68.03a	74.53a	96.97a	112.22a		
25	79.81a	88.06a	113.64a	124.89a		
50	122.31a	133.56a	132.20a	143.45a		
75	174.52a	185.77a	152.43a	163.68a		
100	227.81a	239.06a	171.25a	192.50a		
CD	726.67***	979.18***	46.63***	55.64***		
F-calculated	7.36	6.34	9.19	8.42		

Table 6. Content of anions of soil after irrigation of *V. radiata* with DE till harvesting (90 days after sowing) in both seasons.

Least square mean analysis; Significant F -***P >0.1% level; a - significantly different to the control; CD- Critical difference.

Table 7. Content of metals in soil after irrigation of V. radiata with distillery effluent till harvesting (90 days after sowing) in both seasons.

%DE ×	Zn (n	ng Kg ⁻¹)	Cd (mg Kg ⁻¹)		Cu (m	Cu (mg Kg ⁻¹)		Mn (mg Kg ⁻¹)		Cr (mg Kg ⁻¹)	
Season	Rainy	Summer	Rainy	Summer	Rainy	Summer	Rainy	Summer	Rainy	Summer	
0 (Control)	0.79	0.81	0.11	0.11	2.02	2.05	0.18	0.19	0.14	0.18	
5	1.19	1.36	0.15	0.17	2.22	2.37	0.41	0.48	0.73	0.98	
10	1.43a	1.55a	0.42a	0.55a	3.43	3.65	1.46	1.54	0.94	1.39	
25	2.50a	2.63a	0.68a	0.43a	5.07a	5.99a	1.98a	2.61a	1.27a	1.62a	
50	2.97a	3.29a	1.29a	1.68a	6.69a	6.87a	2.78a	3.85a	1.98a	2.33a	
75	3.39a	3.75a	1.84a	1.97a	9.44a	10.47a	3.26a	4.26a	2.46a	2.53a	
100	3.94a	4.37a	2.42a	2.58a	10.89a	11.47a	4.79a	5.84a	2.89a	3.17a	
CD	38.02***	43.88***	3.03***	3.12*	123.32***	127.97***	14.16***	14.1***	53.53***	49.21***	
F-calculated	0.51	0.47	0.014	0. 21	0.93	0.91	0.08	0.09	0.23	0.24	

Least square mean analysis; Significant F -***P >0.1% level,*P> 5% level; a - significantly different to the control; CD- Critical difference; ns - Not Significant.

It has also been reported that the higher values of the EC of DE irrigated soil indicated enrichment of soluble cations and anions such as Na⁺, K⁺, Ca⁺, Mg²⁺, Cl⁻, PO₄³⁻ and SO₄²⁻ through the use of effluent in different concentrations (Patterson et al., 2008; Kumar and Chopra, 2012). In the present study, there was a maximum increase in the EC (3.94-4.36 dS m⁻¹) of soil as the concentration of DE increased up to 100%. This might be due to increase concentration of potassium salts which are mainly responsible for increasing the EC of the DE as also reported by Chandra et al. (2008).

The ANOVA showed that 25 to 100% concentrations of DE showed significant (P<0.05) changes in moisture content, WHC, OC, Na⁺, K⁺, Ca²⁺, Mg²⁺, Fe²⁺, TKN, PO4³⁻, SO4²⁻, Zn, Cd, Cu, Cr and Mn of the soil in both the rainy and summer season (Tables 3 to 7). More significant (P<0.001) changes were also recorded in OC, Na⁺, K⁺, Ca²⁺, Mg²⁺, Fe²⁺, TKN, PO4³⁻, SO4²⁻, Zn, Cd, Cu, Cr and Mn with 10 to 100% concentration of DE. Non significant (P>0.05) change was found in BD in both the cultivated

seasons (Tables 3 to 7). The coefficient of correlation (rvalue) on soil characteristics revealed that EC, OC, Na⁺, K⁺, Ca²⁺, Mg²⁺, Fe²⁺, TKN, PO₄³⁻, SO₄²⁻, Zn, Cd, Cu, Mn and Cr were positively correlated with all DE concentrations (Table 8). Moisture content (r = -0.95), WHC (r = -0.96), BD and pH (r = -0.97 and r = -0.98) shown their negative correlation were with all concentrations of DE. Moreover, the contamination factor (Cf) indicated that the contamination of metals in the soil was found in order of Mn>Cd>Cr>Cu>Zn after irrigation with DE in both the rainy and summer season (Figure 1). Among various metals, Mn (79.93 and 80.25) showed the maximum contamination factor; while Zn (4.13 and 4.22) showed the minimum with 100% concentration of DE irrigated soil in both the rainy and summer season. These findings were very much in accordance with the earlier reported results by Chandra et al. (2009).

pH is an important parameter as many nutrients are available only at a particular range of pH for plant uptake. The earlier studies reported that a pH range of 6.0 to 8.3

DE/soil characteristics	Season	r - value
DE versus soil EC	Rainy	+0.98
	Summer	+0.98
DE versus soil OC	Rainy	+0.99
DE Versus son OC	Summer	+0.99
DE versus soil Na⁺	Rainy	+0.98
DE Versus soli iva	Summer	+0.98
DE versus soil K⁺	Rainy	+0.64
	Summer	+0.64
DE versus soil Ca ²⁺	Rainy	+0.75
	Summer	+0.75
DE versus soil Mg ²⁺	Rainy	+0.62
	Summer	+0.62
DE versus soil TKN	Rainy	+0.99
	Summer	+0.99
DE versus soil PO4 ³⁻	Rainy	+0.99
	Summer	+0.99
DE versus soil SO4 ²⁻	Rainy	+0.96
	Summer	+0.96
DE versus soil Fe ²⁺	Rainy	+0.99
	Summer	+0.99
DE versus soil Zn	Rainy	+0.93
	Summer	+0.93
DE versus soil Cd	Rainy	+0.97
	Summer	+0.98
DE versus soil Cu	Rainy	+0.99
	Summer	+0.99
DE versus soil Mn	Rainy	+0.99
	Summer	+0.99
DE versus soil Cr	Rainy	+0.99
	Summer	+0.99

Table 8. Coefficient of correlation (r) between DE and soil characteristics in both seasons.

enhanced the nutrient availability for the growth of plants, and change in pH beyond this limit (below 6.0 and above 8.3) inhibited the availability of nutrients for the growth of plants (Charman and Murphy, 1991). In the present study, pH of the soil was recorded to be within range, that is, 6.27-6.30 at 100% DE concentration that may help in enhancing the various nutrients of the soil. The higher concentration of Na⁺ in soil after effluent irrigation may be associated with the presence of high concentration of bicarbonates in the effluent (Hati et al., 2007). The concentration of Cl⁻ and SO₄²⁻ was also recorded to be higher in soil in irrigation with 100%



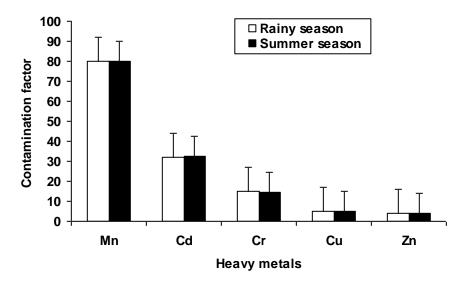


Figure 1. Contamination factor of the metals in soil in the summer and rainy season after fertigation with DE.

concentration of DE. Earlier studies revealed that effluent irrigation adds a significant quantity of salts to the soil such as sulfates and chlorides that stimulate the growth at lower concentration, but inhibits the growth at higher concentrations. The occurrence of Cl⁻ ions in DE increases with the increase in EC. These two anions significantly contributed towards the salinity hazards associated with irrigation water (Joshi et al., 2000), who reported that Cl⁻ and SO₄²⁻ were relatively higher in fields that received distillery effluent.

The content of K⁺, Ca²⁺, Mg²⁺ and PO₄³⁻ contents were noted higher in the soil. This might be due to increase in mineralization activity of organic matter, as well as nutrients present in DE that may be responsible for increased availability of plant nutrients. Earlier studies on these parameters also showed an increase of these parameters in experimental soil irrigated with distillery effluent (Kaushik et al., 2005). In case of metals, the content of Zn, Cd, Cu, Mn and Cr in the soil were increased with the increase in DE concentrations (Table 8). This might be due to more amount of organic matter in the soil, which provides the more binding sites to the metals (Sauve et al., 2000).

Agronomic characteristics

Effect on germination of V. radiata

During the present study, at germination stage, that is, at 0-15 day's seed emergence period of *V. radiata* was 4.67 days with 100% concentration of DE in both the rainy and summer seasons. It was found to be non-significantly (P>0.05) and negatively correlated (r = -0.96) with different concentrations of DE in both the cultivated seasons. The maximum seed germination (94.67% and

96.00%) of V. radiata was recorded with control irrigation, while the minimum germination (78.33% and 86.00%) was found with 100% concentration of DE. It was recorded to be negatively correlated (r = -0.97 and r = -0.98) with different concentrations of DE in both the cultivated seasons. The 50%, 75% and 100% concentrations of DE had significant (P<0.05) effect on seed germination in both seasons. The germination was also recorded to be significantly (P<0.01) different by 25% to 100% concentration of DE in summer season. The maximum relative toxicity of the DE against the seed germination of V. radiata (10.41% and 17.25%) was found with 100% concentration of DE, and it was positively correlated (r = +0.74 to +0.88) with DE concentrations in both seasons. Pandey et al. (2007) also reported that the germination percentage of Wheat (T. aestivum L.), Pea (P. sativm L.) and Lady's Finger (A. esculentus L.) decreases with increasing concentration of effluent in all the tested seeds, where as the germination speed and germination value increases from control to 25 and 50% concentration, and decreases from 50 to 75% and 100% of distillery effluent. Kannan and Upreti (2008) also found that the percentage of germination and speed of germination in Mung bean (Vigna radiata, L.R. Wilczek) were significantly concentration-dependent, and were declined in untreated effluent irrigation.

This type of germination pattern of *V. radiata* is likely due to the presence of toxicants in higher concentration of effluent, which may inhibit the germination at higher concentrations. In addition, it is also due to the fact that the availability, uptake and leaching of nutrients are greatly influenced by a number of physico-chemical factors. Among various physico-chemical factors, the pH plays a significant role in the soil. In the acidic soil environment, the availability of the basic cations like Ca²⁺,

%DE × Season		Shoot length (cm)		Root length (cm)		Dry wt. (g)		Chlorophyll content (mg./g.f.wt)		LAI	
Season		Rainy	Summer	Rainy	Summer	Rainy	Summer	Rainy	Summer	Rainy	Summer
0 (Control)		36.73	35.06	13.21	12.51	9.08	9.24	2.82	2.81	3.28	3.26
5		44.71	40.87	15.11	14.77	12.51	11.37	3.61	3.05	3.81	3.71
10		48.83	43.00	17.31	16.14	14.01	12.84	3.86	3.39	3.96	3.76
25		56.66	62.66a	25.29	26.11a	16.75	16.58 a	3.98	3.95a	4.23	4.36a
50		68.57a	55.74	29.31a	25.29	18.29a	14.99	4.73a	3.72	4.86a	4.15
75		59.58	52.24	27.46	24.19	17.16	14.12	4.34	3.56	4.56	4.08
100		52.57	49.40	19.71	18.54	15.45	13.28	3.89	3.37	3.80	3.68
CD		14.19	13.54	8.3	9.47	6.64	6.39	1.3	1.02	1.05	1.05
F-calculated		3.34*	3.71**	3.00*	2.80*	2.80*	3.32*	2.67*	4.83**	2.59*	2.57*

Table 9. Effect of DE irrigation on vegetative growth stage (45 days) of V. radiata in both seasons.

Least square mean analysis; Significant F -**P >1% level,*P> 5% level; a - significantly different to the control; CD- Critical difference; ns - Not Significant.

Mg²⁺, K⁺ and Na⁺ becomes lower due to leaching. Thus, the availability of these nutrients decreases as per increase in the acidic character of the soil. However, it directly affects the germination and growth of the crop plants (Charman and Murphy, 1991; Patterson et al., 2008). Similarly, salts are usually most damaging to young plants, but not necessarily at the time of germination, although the high salt concentration can slow seed germination by several days or completely inhibit it. The soluble salts move readily with water and the evaporation moves salts to the soil surface where they accumulate, and make the soil surface harden. As a result there might be delay in seed germination (Kaushik et al., 2005).

Effect on vegetative growth stage

At vegetative growth stage, that is, at 45 days, the maximum shoot length, root length, dry weight, chlorophyll content and LAI/plant of V. radiata were recorded with 50% concentration of DE in the rainv season, while in the summer season it was recorded with 25% concentration of DE (Table 9). The ANOVA indicated that the concentrations of DE had significant (P<0.05) effect on shoot length, root length, dry weight, chlorophyll content and LAI/plant of V. radiata in both cultivated seasons. Shoot length and chlorophyll content of V. radiata were also found to be more significantly (P<0.01) different in the summer season. The 50% concentration of DE showed significant (P<0.05) effect on shoot length, root length, dry weight, chlorophyll content and LAI/plant of V. radiata in the rainy season while 25% concentration of DE showed significant (P<0.05) effect in the summer season. Shoot length of V. radiata was also found more significantly (P<0.01) different with 50% concentration of DE in summer season. These observations were in agreement with the earlier reported results by Chandra et al. (2008). Tharakeshwari and Jagannath (2011a) also reported that 25% concentration of distillery effluent was found to be beneficial for the growth of shoot and root in *V. radiata* (L.) and *V. unguiculata* (L.) plants as compared to control, indicating the enhancing influence of plant nutrients present in the effluent. Bharagava et al. (2008) also found that post distillery effluent irrigation increases the chlorophyll and protein contents in Indian mustard plants (*Brassica nigra* L.) at the lower concentrations (25% and 50%) of DE followed by a decrease at higher concentrations (75% and 100%) of DE as compared to their respective controls.

At vegetative growth stage, the growth of V. radiata showed insignificant and positive correlation with different concentration of DE (Table 10) and it was decreased at higher concentration of DE. The higher EC is the indicator of higher salt content in higher concentrations of DE. The high salt content acts as a limiting factor for the seed germination and vegetative growth. Thus, the higher EC has been found to inhibit for the plant growth. These findings suggest a link between EC and plant growth as has earlier been reported by Patterson et al. (2008). The chlorophyll content was observed higher by 50% DE in rainy season and by 25% in summer season. It is likely due to the presence of significant content of Fe. Mg and Mn in the DE, which is associated with the synthesis of chlorophyll in plants. The deficiency of Fe, Mn and Mg in plants causes significant changes in the plant metabolism, and induces chlorosis and necrosis, early leaf fall and low reutilization as earlier reported by Porra (2002).

Effect on flowering and fruiting stage

At flowering and fruiting stage, that is, at 60 days, the number of flowers showed insignificant (P>0.05) and

DE/ V. radiata	Season	r - value
DE voreus shoot longth	Rainy	+0.25
DE versus shoot length	Summer	+0.25
DE versus root length	Rainy	+0.31
	Summer	-0.21
DE versus dry weight	Rainy	+0.26
	Summer	+0.25
DE versus chlorophyll content	Rainy	+0.19
	Summer	+0.23
DE versus LAI	Rainy	+0.16
	Summer	+0.15
DE versus no. of flowers/plant	Rainy	+0.16
	Summer	+0.27
DE versus no. of pods	Rainy	+0.21
	Summer	+0.22
DE versus crop yield	Rainy	+0.39
	Summer	+0.47
DE versus HI	Rainy	+0.10
	Summer	+0.58
DE versus Zn	Rainy	+0.99
	Summer	+0.99
DE versus Cd	Rainy	+0.95
	Summer	+0.96
DE versus Cu	Rainy	+0.99
	Summer	+0.99
DE versus Mn	Rainy	+0.98
	Summer	+0.98
DE versus Cr	Rainy	+0.99
	Summer	+0.99

Table 10. Coefficient of correlation (r) between distillery effluent and V. radiata in both seasons.

positive correlation with different concentrations of DE (Table 10). Maximum flowers and pods per plant of *V. radiata* were recorded with 50% concentration of DE in rainy season while with 25% concentration of DE in summer season (Table 11). The concentrations of 10 to 100% DE had significant (P<0.05) effect on the number of flowers and pods per plant in both the rainy and

summer seasons. Thus, 50% dilution of DE in the rainy season and 25% dilution of DE in the summer season were found suitable for maximum flowering and fruiting of *V. radiata*. Nitrogen and phosphorus are essential for flowering. Too much nitrogen can delay or prevent flowering, while phosphorus deficiency is sometimes associated with poor flower production or flower abortion.

	Flower	ing and fruiti	ng stage (6	0 days)		Maturity sta	age (90 days)
%DE × Season	No. of flowers		No. c	of pods	CY/pl	ant (g)	t (g) HI (%)	
_	Rainy	Summer	Rainy	Summer	Rainy	Summer	Rainy	Summer
0(Control)	20.36	20.12	18.33	18.00	42.59	42.51	488.43	460.06
5	23.56	21.24	20.33	19.17	55.09	48.92	524.16a	481.02a
10	25.86a	22.96a	22.83a	19.67a	58.34a	54.34a	529.88a	501.29a
25	27.47a	35.88a	25.17a	31.83a	78.57a	78.91a	513.86a	521.89a
50	38.98a	31.87a	34.17a	28.00a	82.24a	74.70a	537.22a	509.34a
75	34.63a	28.56a	30.99a	25.33a	76.80a	72.94a	514.12a	507.07a
100	30.85a	25.14a	27.72a	23.00a	68.61a	62.95a	511.87a	505.74a
CD	3.34	3.10	3.12	3.97	10.57	10.29	15.62	14.56
F-calculated	2.98*	2.84*	2.79*	2.75*	3.50**	4.13**	2.89*	3.45**

Table 11. Effect of DE irrigation on flowering and fruiting (60 days) and maturity stage (90 days) of V. radiata in both seasons.

Least square mean analysis; Significant F -**P >1% level, *P> 5% level; a - significantly different to the control; CD- Critical difference; ns - Not Significant.

The maximum flowering was observed with the 50% DE in rainy season and 25% DE in summer season. This might be due to the fact that these concentrations contain sufficient N and P. Furthermore, P and K prevent flower abortion so pod formation occurs (EI-Naggar, 2005).

Effect on maturity stage

At maturity stage, that is, at 90 days, DE concentrations of 5% to 100% showed significant effect on crop yield and harvest index in both the rainy and summer seasons (Table 10). Maximum crop yield/plant and harvest index (HI) of V. radiata were recorded with 50% concentration of DE in the rainy season while in the summer season the maximum was noted with 25% concentration of DE (Table 11). Crop yield and HI of the plant were recorded significantly (P<0.05) and are affected in both the cultivated seasons. Crop yield and HI were also found more significantly (P<0.01) different with different concentrations of DE in the summer season. The number of pods, crop yield and HI showed insignificant (P>0.05) and positive correlation with different concentrations of DE (Table 11). The role of K, Fe, Mg and Mn at maturity is important and associated with synthesis of chlorophyll and enhances the formation of pods at harvest (El-Naggar, 2005; Naeem et al., 2006). The K, Fe, Mg and Mn contents could benefit pod formation and yield of the plant as it does for soybean (Glycine max L.) as reported by Hati et al. (2007). The 25% distillery effluent favoured pod formation and crop yield of V. radiata. This is likely due to the presence of K, Fe, Mg and Mn contents in 25% and 50% distillery effluent; higher distillery effluent concentrations lowered pod formation and crop yield of V. radiata.

Micronutrients in V. radiata

In the present study, ANOVA showed that the

concentrations of 25% to 100% of DE had significant (P<0.001) effect on the content of Zn, Cd, Cu, Mn and Cr in *V. radiata* (Figures 2, 3 and 4). It is likely to occur due to the presence of significant quantities of these metals in the DE and irrigated soil due to more irrigation frequency with increase in irrigated concentrations (Kumar and Chopra, 2010). The content of Zn, Cd, Cu, Mn and Cr in *V. radiata* was recorded maximum with 100% DE (Figures 2, 3 and 4). The r value of metals, Zn, Cd, Cu, Mn and Cr in *V. radiata* were shown their positive correlation with concentrations of DE in both the rainy and summer season (Table 10).

In addition, contamination factor (Cf) indicated the contamination of metals in V. radiata after DE irrigation. The (Cf) of various metals was in order of Cr>Cu>Zn>Cd>Mn in V. radiata after irrigation with DE in both the cultivated seasons (Figure 5). The maximum contamination factor was found for Cr (22.07 and 22.27), while the minimum was found for Mn (11.63 and 12.30) in V. radiata with 100% concentration of DE in both cultivated seasons. These findings suggest a link between higher content of metals and higher soil organic matter which provides more binding sites for metal accumulation in the soil and plants as has been reported by Sauve et al. (2000). Chandra et al. (2009) reported the high metals (Cu, Cd, Cr, Zn, Fe, Ni, Mn, and Pb) content in wheat and mustard plants irrigated with mixed distillery and tannery effluents. Pandey et al. (2008) observed that the distillery effluent contains potentially hazardous toxic heavy metals (Cd, Cr, Ni and Zn) which affect the seed germination and seedling growth of maize (Zea mays L.) and rice (Oryza sativa L.).

Conclusions

The present study concluded that the DE was rich in the plant nutrients and heavy metals. The DE parameters namely: BOD, COD, Cl⁻, Ca²⁺, TKN, $NO_3^{2^-}$, $SO_4^{2^-}$, MPN

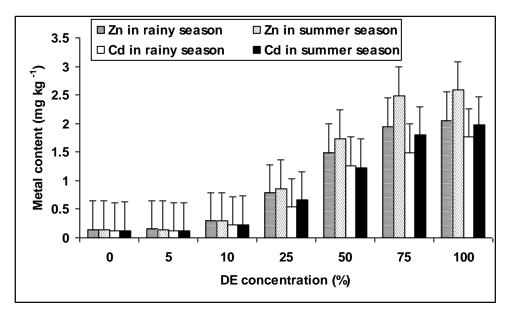


Figure 2. Content of Zn and Cd in *V. radiata* in the summer and rainy season after fertigation with DE.

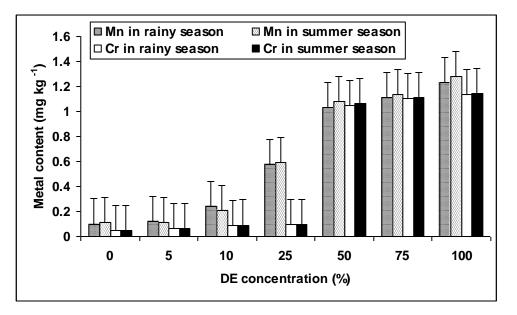


Figure 3. Content of Mn and Cr in *V. radiata* in the summer and rainy season after fertigation with DE.

and SPC were found beyond the prescribed limit of Indian Irrigation Standards. The DE irrigation significantly increased the OC, Na⁺, K⁺, Ca²⁺, Mg²⁺, Fe²⁺, TKN, PO₄³⁻, SO₄²⁻, Zn, Cd, Cu, Mn and Cr content of the soil. The agronomical performance of *V. radiata* was gradually increased from 5 to 50% concentrations of DE in the rainy season and 5 to 25% concentration of DE in the summer season, while it was decreased at higher concentration, that is, from 75 to 100% concentrations of

DE in the rainy season and 50 to 100% concentrations of DE in the summer season in comparison to the control. The maximum growth of *V. radiata* at maturity stage was observed at 50% concentration of DE in the rainv season and at 25% in the summer season. The contamination various factor of metals were in order of Mn>Cd>Cr>Cu>Zn for soil and Cr>Cu>Zn>Cd>Mn for V. radiata in both seasons after irrigation with DE. Thus, DE fertigation improved the soil nutrient status in both

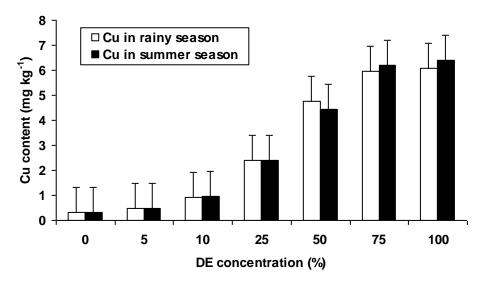


Figure 4. Content of Cu in *V. radiata* in the summer and rainy season after fertigation with DE.

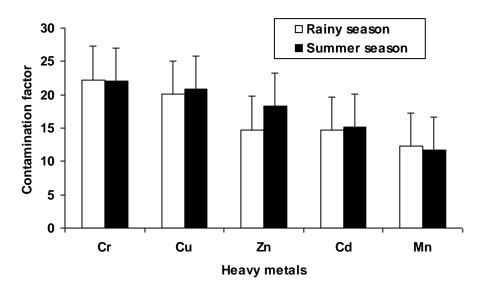


Figure 5. Contamination factor of various metals in *V. radiata* in the summer and rainy season after fertigation with DE.

cultivated seasons. Therefore, there is certain growth stimulating as well as inhibiting substances present in the DE, which are responsible for this growth pattern. Accordingly, this agro-based effluent has potentiality in the form of plant nutrients needed by *V. radiata* crop plant. It can be used for irrigation purposes after appropriate dilution, which can be one of the solutions for combating environmental pollution and to meet the challenge of water scarcity for irrigation purposes. Moreover, further research is required for the use of distillery effluent in the cultivation of *V. radiata* with different soil types and bio-chemical changes in crop plants after fertigation with distillery effluent.

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